MULTI-LAYER AIRWAY ORGANOIDS AND METHODS OF MAKING AND USING THE SAME

RELATED APPLICATIONS

[0001] This application is a divisional of U.S. application Ser. No. 15/768,100 filed Apr. 13, 2018, now allowed, which is a 35 U.S.C. § 371 national stage application of PCT International Application No. PCT/US2016/056947, filed on Oct. 14, 2016, which claims priority from U.S. Provisional Patent Application Nos. 62/242,611, filed Oct. 16, 2015, and 62/404,931, filed Oct. 6, 2016, the contents of which are incorporated herein by references in their entireties. The above-referenced PCT International Application was published in the English Language as International Publication No. WO 2017/066507 A1 on Apr. 20, 2017.

FIELD OF THE INVENTION

[0002] This invention relates generally to multi-layer airway organoids and methods of making and using of the same.

BACKGROUND OF THE INVENTION

[0003] The study of respiratory infection is significantly limited by a lack of suitable in vivo and in vitro models to investigate interactions between the respiratory epithelium, infection and disease. For example, animal models often do not acquire the pathological abnormalities in the airways and lungs seen in humans. See, e.g., Guilbault et al., *Cystic fibrosis mouse models,* American Journal of Respiratory Cell and Molecular Biology. 2007; 36(1):1-7. Additionally, most in vitro models are unable to create the differentiated tissue components and structural complexity of the airway epithelium. See, e.g., Lang et al., *Three-dimensional culture of hepatocytes on porcine liver tissue-derived extracellular matrix,* Biomaterials. 2011; 32(29):7042-7052.

[0004] Primary airway epithelial cells, derived from cadaver tissue and expanded ex vivo on 2D plastic culture surfaces, remain the current standard for disease modeling and therapy evaluation in vitro. However, these techniques present cells with artificial conditions, including two-dimensional (2D) growth surfaces that are several magnitudes stiffer than most soft tissues, and lack of important signals from the tissue microenvironment. As a consequence, they impose a selective pressure on the cells that substantially alter their heterogeneity and functional properties. See, e.g., Anderson et al., *Tumor morphology and phenotypic evolution driven by selective pressure from the microenvironment,* Cell 127.5 (2006): 905-915. For example, plastic culture expanded cells often become non-ciliated, a significant limitation in studying bacterial pathogens of the airways, which often display preferential attachment to ciliated respiratory epithelium in vivo. See, e.g., Matsui et al., *Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease.* Cell. 1998; 95(7):1005-1015; Gray et al., *Mucociliary differentiation of serially passaged normal human tracheobronchial epithelial cells.* American Journal of Respiratory Cell and Molecular Biology. 1996; 14(1):104-112. The lack of physiological airway models represents a significant limitation to the study of the pathogenesis of infection in the airway.

[0005] US Patent Application Publication 2009/0227025 to Nichols et al. discussed the use of progenitor or stem cells to generate new lung tissue in an in vitro system using microgravity conditions. U.S. Pat. No. 8,647,837 to Mahmood et al. and U.S. Pat. No. 5,750,329 to Quinn et al. discuss the use of alveolar and endothelial cell layers in an artificial tissue construct for the study of lung diseases concerning the alveoli, or air sacs, of the lungs. U.S. Pat. No. 8,338,114 to Goodwin discusses three-dimensional (3D) human broncho-epithelial tissue-like assemblies produced in a rotating wall vessel with microcarriers by co-culturing mesenchymal bronchial-tracheal cells and bronchial epithelium cells. However, there remains a need for improved in vitro systems that can be used for study of infection and pathogenesis affecting the lungs.

SUMMARY OF THE INVENTION

[0006] Provided herein is an artificial mammalian lung organoid, comprising:

[0007] (a) an epithelial cell layer comprising mammalian lung epithelial cells;

[0008] (b) a stromal cell layer comprising mammalian lung fibroblast cells; and

[0009] (c) an endothelial cell layer comprising mammalian endothelial cells (e.g., microvascular endothelial cells).

[0010] In some embodiments, the organoid further comprises a porous membrane (e.g., a polymeric material) between said epithelial cell layer and said stromal lung cell layer and/or between said stromal lung cell layer and said endothelial lung cell layer.

[0011] In some embodiments, the cells of the lung epithelial cell layer are polarized. In some embodiments, the cells of the lung endothelial cell layer, stromal cell layer and/or epithelial cell layer are human.

[0012] In some embodiments, the lung organoid is an upper airway lung organoid. In some embodiments, the mammalian lung epithelial cells are bronchial epithelial cells. In some embodiments, the mammalian lung epithelial cells comprise normal bronchial epithelial cells. In some embodiments, the mammalian lung epithelial cells comprise diseased bronchial epithelial cells.

[0013] In some embodiments, the bronchial epithelial cells comprise basal, goblet, ciliated and/or clara cells.

[0014] In some embodiments, the ratio of mammalian lung fibroblast cells of the stromal layer and mammalian endothelial cells of the endothelial cell layer is from 2:1 to 1:2. In some embodiments, the ratio of mammalian lung fibroblast cells of the stromal layer and mammalian epithelial cells of the epithelial cell layer is from 2:1 to 1:2. In some embodiments, the ratio of mammalian endothelial cells of the endothelial layer and mammalian epithelial cells of the epithelial cell layer is from 2:1 to 1:2.

[0015] In some embodiments, the porous membrane is coated on one or both sides with laminin, collagen type I, collagen type IV, fibronectin, elastin, a lung tissue-derived extracellular matrix composition, or a combination thereof.

[0016] In some embodiments, the lung organoid is infected with a lung pathogen. In some embodiments, the lung organoid is infected with *Bordetella pertussis* or *Pseudomonas aeruginosa.*

[0017] Also provided is a microfluidic device comprising the lung organoid as taught herein. The microfluidic device may include a housing comprising a chamber and a channel. The lung organoid may be in the chamber, the channel may